PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION See Form PCT/IPEA/416	
40971		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/FI2004/000228	14.04.2004	14.04.2003
International Patent Classification (IPC) or national classification and IPC		
C12N 15/90, C12N 15/79		
Applicant		
Finnzymes Oy et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of _____ sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☒ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☒ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11.02.2005	13.07.2005
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Form PCT/IPEA/409 (cover sheet) (January 2004)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI2004/000228

Box No. I Basis of the report

1.- With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))

☐ publication of the international application (under Rule 12.4)

☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:

pages 1-33 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

pages _____ as originally filed/furnished

pages* _____ as amended (together with any statement) under Article 19

pages* 1-2 received by this Authority on 06.06.2005

pages* _____ received by this Authority on _____

☒ the drawings:

pages 1-8 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

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Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

The priority claim is considered valid. Document US2003143740 A1 is therefore not considered herein.

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Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-11	YES
	Claims		NO
Inventive step (IS)	Claims	1-11	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-11	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents are considered relevant:

D1) US2002/0132350 A1
D2) Lamberg et al: "Efficient insertion mutagenesis strategy for bacterial genomes involving electroporation of in vitro-assembled DNA transposition complexes of bacteriophage mu", Appl. Environ. Microbiol. 2002 Feb;68(2):705-12
D3) Goryshin et al: "Insertional transposon mutagenesis by electroporation of released Tn5 transposition complexes", Nat Biotechnol. 2000 Jan;18(1):97-100
D4) Shi et al: "Efficient transposition of preformed synaptic Tn5 complexes in Trypanosoma brucei", Mol Biochem Parasitol. 2002 Apr 30;121(1):141-4
D5) US6294385 B1
D6) Schagen et al: "Towards integrating vectors for gene therapy: expression of functional bacteriophage MuA and MuB proteins in mammalian cells", Nucleic Acids Res. 2000 Dec 1;28(23):E104. (enclosed by the applicant)

D1 shows a method for targeted genetic manipulation of e.g. maize and soybean cells. Active cleaved donor complex (CDC) comprising Mu sequences and MuA is transformed into maize and soybean cells by microprojectile bombardment. The CDC inserted into the cell has the intact MuA tetrameric core attached. Reporter genes and nuclear localisation sequences can be included. See Figure 4, [0038], [0041] lines 1-3, [0042], [0046]-[0047] line 9, [0058], [0100] lines 1-14, [0156], p. 24-25 examples 6-7 and [0188].

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

D2 shows the assembly of integration-proficient Mu transposition complexes that, after introduction into bacterial cells by electroporation, execute transposon integration into bacterial chromosomes. It is stated that the strategy disclosed therein also could be "applicable to gram-positive bacteria and perhaps to some eukaryotic organisms (such as yeast) as well". See abstract, p. 705 right col. last paragraph- p. 707 left col. paragraph 1 and p. 711 left col. paragraph 3-4.

D3 shows that premade Tn5 synaptic complexes can transpose in the yeast Saccharomyces cerevisiae. This is shown by electroporation of a Tn5 transposome into S. cerevisiae. See abstract and p. 99 left col. paragraphs 5 and 7.

D4 shows that in vitro preformed Tn5 synaptic complexes can insert into the genome of T. brucei. See abstract, p. 141 left col. paragraph 2-right col. paragraph 2, p. 142 figure 1B and p. 143 left col. paragraph 2.

D5 shows synaptic Tn5 complexes, formed in vitro, delivered into target cells. Libraries of cells are contemplated. It is stated that no scientific impediment is known to exist that would prevent use of the method in e.g. plant and animal cells. See col. 2 lines 50-59, col. 2 line 66-col. 3 line 4, col. 3 line 63-col. 4 line 4 and col. 8 lines 32-40.

The present application relates to the introduction of in vitro-assembled DNA transposition complexes into eukaryotic cells such as mammalian cells. One benefit is that there is no need to generate an expression system of the transposition machinery for the organism of interest.

Document D1 is considered to represent the closest prior art.

The difference between claim 1 and D1 is that a mammalian cell is targeted. In D1 the target cells are maize and soybean cells. The technical effect achieved by claim 1 is a Mu transposition based method for incorporating target nucleic acid segments to the genome of a mammalian target cell. The problem to be solved is providing a Mu transposition based method for incorporating target nucleic acid segments to the genome of a mammalian target cell.

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

Examples 6-7 of D1 disclose detailed but only suggestive instructions of how Mu complexes are delivered into maize and soybean cells. Actual results are not shown. I.e. no real evidence is given in D1 that MuA is active inside a plant cell.

In D2; the target cell is a bacterium. It is stated that the strategy disclosed therein also could be "applicable to gram-positive bacteria and perhaps to some eukaryotic organisms (such as yeast) as well". No evidence of application in eukaryotic organisms is shown.

At the time the invention was made, the prior art contained no evidence of successful transformation of higher eukaryotes by Mu transposition complexes; only speculative plans suggesting the possibility to do so. Thus, it is considered that prior art contains only a plan to transform higher eukaryotic cells with in vitro-assembled transposition complexes of Mu. No evidence is given showing that it actually works.

In D3, in vitro assembled Tn5 synaptic complexes are shown to transpose in the yeast Saccharomyces cerevisiae, and in D4 in Trypanosoma brucei. Thus, D3 and D4 show that Tn5 based transposition is possible inside a monocellular lower eukaryote; and consequently shows that a bacterial transposition system can work in a lower eukaryotic cell.
The difference between the invention according to claim 1 and D3 or D4 is that Mu transposition complexes are used in stead of Tn5 complexes, and that the target cell is a mammalian cell in claim 1.
Even though the skilled person knows that a common DNA transposition mechanism is shared among a variety of mobile elements, the mechanisms are not exactly identical. D3 and D4 show that Tn5 bases transposition is possible inside a monocellular lower eukaryote (a yeast and T. brucei). However, it is considered that these results cannot be generalized to concern all eukaryotes including multicellular organisms such as mammalian cells. Thus, D3 and D4 are not motivation for the skilled person to try another transposon (MuA) in very different organisms (mammalian cells) compared to the ones used in D3 or D4.

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

In D5, in vitro assembled Tn5 synaptic complexes are delivered into target cells. In the example, E. coli is transformed, but it is stated that no scientific impediment is known to exist that would prevent use of the method in e.g. plant and animal cells. Thus, the difference between claim 1 and D5 is that in claim 1, mammalian cells are the target, and Mu is used. However, in the same way as argued above, the disclosure of D5 is not considered as motivation for the skilled person to try another transposon (MuA) in very different organisms (mammalian cells) compared to the one used in D5. In D5, the application in plant and animal cells is merely speculated and no examples or evidence is shown.

D6 (enclosed by the applicant) shows an expression vector based transposition system in mammalian cells. The system is based on MuA and MuB proteins and mammalian cells are co-transfected with a donor construct and the vector containing MuA and MuB. No Mu-specific integrations are detected in the transformed cells. It is stated that it is questionable whether an active Mu transposase can be established in mammalian cells (see the abstract and the last paragraph of the discussion p. 6). Therefore, if the skilled person would have been motivated by the suggestions made in prior art to try the transformation of mammalian cells with a transposition complex, it is not likely that she would have chosen a MuA transposition based system for the experiment.

Consequently, the invention according to claim 1 is considered to involve an inventive step since, in view of prior art, it was not expected that MuA is capable of catalysing transposition in mammalian cells.
The invention according to claims 1-11 is novel and is considered to involve an inventive step.

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Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
US2003/0143740	31/07/2003	15/10/2002	

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on _____

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

We claim:

1. A method for incorporating nucleic acid segments into cellular nucleic acid of an isolated mammalian target cell, the method comprising the step of:

delivering into the mammalian target cell an *in vitro* assembled Mu transposition complex that comprises (i) MuA transposases and (ii) a transposon segment that comprises a pair of Mu end sequences recognised and bound by MuA transposase and an insert sequence between said Mu end sequences, under conditions that allow integration of the transposon segment into the cellular nucleic acid.

2. The method according to claim 1, wherein said Mu transposition complex is delivered into the target cell by electroporation.

3. The method according to claim 1, wherein the nucleic acid segment is incorporated to a random or almost random position of the cellular nucleic acid of the target cell.

4. The method according to claim 1, wherein the nucleic acid segment is incorporated to a targeted position of the cellular nucleic acid of the target cell.

5. The method according to claim 1, wherein the target cell is a human cell.

6. The method according to claim 1, wherein said animal cell is a mouse cell.

7. The method according to claim 1, wherein said insert sequence comprises a marker, which is selectable in mammalian cells.

8. The method according to claim 1, wherein a concentrated fraction of Mu transposition complexes are delivered into the target cell.

9. The method according to claim 1 further comprising the step of incubating the target cells under conditions that promote transposition into the cellular nucleic acid.

10. A method for forming an insertion mutant library from a pool of mammalian target cells, the method comprising the steps of:

a) delivering into a mammalian target cell an *in vitro* assembled Mu transposition complex that comprises (i) MuA transposases and (ii) a transposon segment that comprises a pair of Mu end sequences recognised and bound by MuA transposase and an insert sequence with a selectable marker between said Mu end sequences, under conditions that allow integration of the transposon segment into the cellular nucleic acid; and

b) screening for cells that comprise the selectable marker.

11. A kit for incorporating nucleic acid segments into cellular nucleic acid of a mammalian target cell comprising a concentrated fraction of Mu transposition complexes with a transposon segment that comprises a marker, which is selectable in mammalian cells.